

## **Association of 17 candidate genes and their combinations with polycystic ovary syndrome**

Running title: 17 candidate genes in PCOS

Joanna Skommer<sup>1</sup>, Seija Korhonen<sup>2</sup>, Maritta Hippeläinen<sup>3</sup>, Juha S. Tapanainen<sup>4</sup>, Seppo Heinonen<sup>5</sup>,  
Markku Laakso<sup>1</sup>

<sup>1</sup> Department of Medicine, University of Kuopio, Kuopio, Finland and Departments of Obstetrics and Gynecology; <sup>2</sup> Central Hospital of Mikkeli, Mikkeli, Finland; <sup>3</sup> University of Kuopio, Kuopio, Finland;

<sup>4</sup> University of Oulu, Oulu, Finland; and <sup>5</sup> Kuopio University Hospital, Kuopio, Finland

### Correspondence and reprint request:

Markku Laakso

Professor and Chair

Department of Medicine

University of Kuopio

70210 Kuopio, Finland

phone:+358-17-172151

fax: +358-17-173993

e-mail: markku.laakso@kuh.fi

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## Abstract

Polycystic ovary syndrome (PCOS) is associated with insulin resistance, obesity and increased risk of type 2 diabetes. Previous studies on the genetics of PCOS have included mostly a small number of patients and investigated only the effects of a single gene and a single polymorphism. In this study we evaluated the association of PCOS with genomic variants and their combinations in 17 genes influencing insulin sensitivity, obesity, type 2 diabetes mellitus, dyslipidemia and/or inflammation in 143 PCOS and 115 control subjects.

Genotype distribution for the 1059 G/C polymorphism of *CRP* differed significantly between the groups ( $p=0.014$ ). The 1059 C allele of *CRP* was associated with a 2.16-fold higher odds ratio [OR] for PCOS (95% confidence intervals 1.02-4.57,  $p=0.044$ ) compared to the 1059 G/G genotype. We also found previously unreported statistically significant interactions between the variants in genes involved in adipocyte function and/or insulin signaling (*PPAR $\gamma$ 2*, *PGC-1 $\alpha$* , *APM1*, *HNF-4 $\alpha$*  and *PC-1*) and their association with PCOS. Our results suggest that both single gene and multiple loci analyses are necessary for the understanding of the genetics of PCOS. We also propose that the combinations of genomic variants in genes related to adipocyte differentiation, regulation of gluconeogenesis and insulin signaling may contribute to the pathogenesis of PCOS.

## **Introduction**

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders among women of reproductive age (1). This highly heterogeneous clinical entity is characterized by biochemical and/or clinical signs of hyperandrogenism, gonadotropin dissociation and irregular menses. Central obesity and dyslipidemia are other typical features in subjects with PCOS. Furthermore, the syndrome is frequently associated with peripheral insulin resistance, hyperinsulinemia and impaired glucose tolerance (2).

The etiology of PCOS is still largely unknown, but it is apparent that insulin resistance is one of the major factors contributing to ovarian hyperandrogenism. Both metabolic abnormalities and androgen excess tend to be more frequent among obese PCOS women than among their normal weight counterparts (3). Weight loss (4) or drugs increasing insulin sensitivity (5) can lead to spontaneous resumption of menses and amelioration of hyperandrogenism in PCOS patients.

Complexity of symptoms in the first-degree relatives of PCOS women, especially cosegregation of insulin resistance and hyperandrogenism, suggest an underlying genetic cause for the disorder (6). Although several lines of evidence show that genetic factors play a major role in the pathogenesis of PCOS, the mode of inheritance of this syndrome remains unclear. Recent studies indicate that PCOS is a complex, polygenic trait determined by interactions between environmental factors and different combinations of common gene polymorphisms (7). Given a variety of clinical presentation, a lack of diagnostic consensus and uncertain etiology of the syndrome, the list of potential candidate genes is long but the probability of finding a single gene variant that substantially contributes to the susceptibility of PCOS is quite low. Therefore, studies on combinations of multiple gene variants are necessary for the understanding of the genetics of this multifactorial disorder.

Most of the previous studies on the genetics of PCOS have included a small number of patients (< 100) and evaluated the association of a single gene or a single polymorphism with PCOS. Multiple genomic variants have been investigated in only one case–control study (8), including only 72 PCOS and 42 healthy women, and no genetic analyses are available on gene-gene interactions in PCOS. A wide range of candidate genes, particularly those related to steroidogenesis, gonadotropin secretion and action, obesity and energy regulation, insulin action, and inflammation (7-11) have been examined in PCOS patients. The following genes analyzed also in this study: adiponectin (*APMI*) (8, 12), interleukin 6 (*IL-6*) (13), tumor necrosis factor (*TNF- $\alpha$* ) (14), calpain 10 (*CAPN10*) (15, 16), peroxisome proliferators-activated receptor  $\gamma$  (*PPAR $\gamma$ 2*) (17) and plasma cell glycoprotein 1 (*PC-1*) (8) have been previously investigated in women with PCOS. Moreover, the Pro12Ala polymorphism of *PPAR $\gamma$ 2* and the Lys121Gln polymorphism of *PC-1* have been shown to increase OR for PCOS in our previous publications on the same cohort (18, 19). In general, the results have not been confirmed in two or more independent studies and therefore no susceptibility genes have been identified for PCOS.

In this study, we analyzed a relatively large number of subjects selected from a genetically homogenous population of Finland. We examined 25 genomic variants in 17 candidate genes related to obesity, insulin resistance, type 2 diabetes mellitus, inflammation and/or dyslipidemia. To our knowledge previous studies have not investigated polymorphisms in the following genes included in this study: upstream stimulatory factor 1 (*USF1*) (20), hepatic lipase (*LIPC*) (21), peroxisome proliferator-activated receptor coactivator 1 (*PGC-1 $\alpha$* ) (22), hepatocyte nuclear factor 4 and 1 (*HNF-4 $\alpha$*  and *HNF-1 $\alpha$* ) (23,24), C-reactive protein (*CRP*) (25), glucagon-like peptide 1 receptor (*GLP1R*) (26), glucose transporter 2 (*GLUT2*) (27), interleukin 1 $\beta$  and 10 (*IL-1 $\beta$*  and *IL-10*) (28,29) and apolipoprotein A (*APOA*). Finally, we investigated gene-gene interactions between different genomic variants and the effects of their combinations on the risk for PCOS.

## Subjects and Methods

### Subjects

Altogether 143 nondiabetic PCOS women were recruited from endocrinology/infertility clinics in the region of Kuopio and Oulu University Hospitals. A total of 115 non-hirsute, fertile women with regular cycles and normal ovaries who delivered at Kuopio University Hospital between January 1999 and December 1999 served as a control group.

In this study the diagnosis of PCOS required the presence of polycystic ovaries on ultrasonography (eight or more subcapsular follicles of 3-8 mm diameter in one plane in one ovary and increased stroma), anovulation and at least one of the following clinical or biochemical disturbances: hirsutism, infertility or hyperandrogenemia (serum total testosterone concentration  $>2.5$  nmol/l or free plasma testosterone  $>40$  pmol/l, evaluated by means of sex hormone-binding globulin and total testosterone assays, and an elevated LH/FSH ratio  $>2$  ). Hirsutism was defined as the presence of excessive body hair in androgen dependent pattern, with a modified Ferriman-Gallwey score of  $\geq 8$  (30). Furthermore, other common causes of anovulation and hyperandrogenism were excluded. Diabetic women were excluded.

All study subjects and controls gave a written informed consent, and the Ethics Committee of the Kuopio University Hospital and the University of Kuopio approved the study protocol.

### Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes using a standard phenol-chloroform extraction method. The UCSNP-43, -44 and -63 variants of *CAPN10*, the Gly482Ser, 3'UTR+2390 G/A (rs3774923) and rs7695542 C/T variants of *PGC-1 $\alpha$* , the -3926 C/T (rs1884614) and the IVS3 +2925 G/A (rs1885088) variants of *HNF-4 $\alpha$* , the Ile27Leu and the Ser487Asn

substitutions of *HNF-1 $\alpha$* , A-250G of *LIPC*, C-174G of *IL-6*, C-511T of *IL-1 $\beta$* , A-1082G of *IL-10*, 1059 G/C of *CRP*, Gly168Ser of *GLP1R*, Thr110Ile of *GLUT2* and 5'UTR C/T (rs3737787) of *USF1* were genotyped using the TaqMan allelic discrimination assay (Applied Biosystems, Foster City, Calif., USA). Briefly, PCR reactions were carried out in a 10  $\mu$ l volume containing 10 ng of genomic DNA, 1X TaqMan Universal PCR Master Mix, each primer at the concentration of 900 nM and each probe at the concentration of 250 nM (primer and probe sequences are available from the authors upon request). The cycling program was a denaturation step at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min using a GenAmp PCR System 2700 (Applied Biosystems). The fluorescence level was measured with an ABI Prism 7000 Sequence Detector (Applied Biosystems), resulting in clear identification of genotypes.

Genotyping of SNP 45 T/G and SNP 276 G/T of the adiponectin gene were performed using SNaPshot primer extension assay and Abi PRISM 3100 Genetic Analyser (Applied Biosystems). PCR and SNaPshot reaction primers and conditions are available from the authors upon request.

For the detection of other genomic variants we performed PCR reactions followed by the single strand conformation polymorphism analysis as reported previously in detail: the Pro12Ala and the 161 C/T polymorphisms of *PPAR $\gamma$ 2* (18, 31), the K121Q polymorphism of *PC-1* (19), and the G-308A polymorphism of *TNF- $\alpha$*  (32).

### **Statistical analysis**

Statistical and power analyses were conducted using Statistical Package for Social Science (SPSS 11.0 software) and the G\*Power software, respectively. The  $\chi^2$  test was applied to examine differences in genotypic distributions and allele frequencies between PCOS and control subjects. In this study the sample size allowed us to detect effect sizes for the difference between frequencies ( $w$ ) of

0.175 for the  $\chi^2$  test with one degree of freedom, and 0.193 for the  $\chi^2$  test with two degrees of freedom (*a priori* power analysis). Sample sizes in the  $\chi^2$  test subgroup analyses permitted the detection of small (0.10-0.30) or moderate (0.30-0.50) effect sizes. Hardy-Weinberg equilibrium was computed based on the expected genotype distribution. Logistic regression analyses were performed to analyze the association of the 1059 G/C polymorphism of *CRP*, *PPAR* $\gamma$ 2 haplotypes and combinations of genomic variants of different genes with PCOS. Frequencies of single nucleotide polymorphism haplotypes were estimated by using the Estimation Haplotype (EH) frequencies software (<ftp://linkage.rockefeller.edu/software/eh>). A value of  $p < 0.05$  was considered statistically significant.

## Results

The clinical characteristics of patients included in our study have been previously published (18, 19). All women with PCOS had irregular menstruation and polycystic ovaries on ultrasonography. Biochemical signs of hyperandrogenism were found in 59% of cases. Compared with the control group, PCOS subjects were significantly more often obese (18, 19).

Altogether 25 variants in 17 genes were examined (Table 1). The distribution of the genotypes for the 1059 G/C polymorphism of *CRP* differed between the groups ( $p=0.014$ ), with the frequency of C allele 9.4% in the PCOS group and 5.8% in the control group ( $p=0.123$ ). Furthermore, we observed a borderline significant difference in the allele distribution of the 276 G/T polymorphism of *APMI* ( $p=0.075$ ).

The distribution of genotype combinations of two *PPAR* $\gamma$ 2 polymorphisms (Pro12Ala and 161 C/T) differed significantly between the PCOS and control groups ( $p=0.010$ ). The number of carriers of the 161 C/C genotype and the 161 T allele was almost significantly different ( $p=0.064$ ) between the groups, stratified according to the Pro12Ala polymorphism of *PPAR* $\gamma$ 2 (Fig.1).

Additionally, we investigated combinations of genomic variants in different genes (Fig.2). The number of carriers of the 276 G/G genotype or the 276 T allele of *APM1* differed significantly between the PCOS and control groups only in subjects having the 3'UTR A allele of *PGC-1 $\alpha$* . Stratification according to the Gly482Ser polymorphism of *PGC-1 $\alpha$*  revealed that number of individuals having the IVS3 G/G genotype or the IVS3 A allele differed only in subjects with the Ser482 allele of *PGC-1 $\alpha$* . Moreover, the frequency of the IVS3 G/G genotype or the IVS3 A allele of *HNF-4 $\alpha$*  differed significantly between PCOS and control women only in carriers of the Lys121Lys genotype of *PC-1*.

In logistic regression analysis carriers of the 1059 C allele of *CRP* had a 2.16-fold higher odds ratio [OR] for PCOS compared to carriers of the 1059 G/G genotype (95% confidence intervals [CI] 1.02-4.57; p=0.044). Furthermore, pooled *PPAR $\gamma$ 2* genotype combinations including both common alleles (Pro12/161C) or both rare alleles (Ala12/161T) were associated with a 5.12-fold higher OR for PCOS compared to subjects having the Pro12/161T or Ala12/161C genotype combinations (95% confidence intervals [CI] 1.07-24.62; p=0.041). The 161 C/C variant increased OR for PCOS in carriers of the Pro12Pro genotype at borderline significance level (p=0.066), whereas in subjects having the Ala12 allele there was an opposite trend (p=0.106) (Table 2).

Additionally, we applied logistic regression analyses to evaluate the contribution of combinations of genomic variants in different genes to PCOS (Table 2). The 276 G/G genotype of *APM1* increased OR for PCOS only in carriers of the 3'UTR A allele of *PGC-1 $\alpha$*  (p=0.039). The IVS3+2925 G/G genotype of *HNF-4 $\alpha$*  was associated with a 2.61-fold increase in OR for PCOS compared to carriers of the IVS3+2925 A allele of *HNF-4 $\alpha$*  only in individuals having the 487Ser allele of *PGC-1 $\alpha$*  (p=0.030). Moreover, the association of the IVS3+2925 G/G genotype of *HNF-4 $\alpha$*  with PCOS was found only in individuals with the protective Lys121Lys genotype of *PC-1* (p=0.018).

Finally, we performed logistic regression analyses to examine possible interactions between different genomic variants (Table 3). Individuals carrying both common genotypes for the 276 G/T polymorphism of *APM1* and the Pro12Ala polymorphism of *PPAR* $\gamma$ 2 had a 2.36-fold higher OR for PCOS compared to carriers of both rare genotypes (p=0.032). The OR was not, however, significantly higher than that for the Pro12Pro genotype of *PPAR* $\gamma$ 2 alone. The interaction between the 3'UTR G/A polymorphism of *PGC-1* $\alpha$  and the 161 C/T polymorphism of *PPAR* $\gamma$ 2 was almost statistically significant (p=0.061 for the interaction term). Individuals with both common genotypes had a 3.89-fold higher OR for PCOS compared to carriers of no risk genotypes (p=0.052) and a 3.56-fold higher OR compared to carriers of at least one risk genotype (p=0.066). Neither of these polymorphisms showed an independent association with PCOS. Furthermore, subjects having both the 3'UTR G/G genotype of *PGC-1* $\alpha$  and the 276 G/G genotype of *APM1* had a 5-fold higher OR for PCOS compared to subjects carrying neither of these risk genotypes (p=0.020). In addition, a combination of the 121Gln allele of *PC-1* and the IVS3+2925 G/G genotype of *HNF-4* $\alpha$  was associated with a 3.5-fold higher OR for PCOS compared to a non-risk genotype combination (p=0.007), and a 2.73-fold higher OR compared to a combination with at least one risk genotype (p=0.009). The interaction between the Gly482Ser polymorphism of *PGC-1* $\alpha$  and IVS3+2925 G/A polymorphism of *HNF-4* $\alpha$  was statistically significant (p=0.035 for the interaction term), but their combination did not modify OR for PCOS in the whole population. Together with the genetic-background dependent effect of the 276 G/A polymorphism of *APM1* and the IVS3+2925 G/A polymorphism of *HNF-4* $\alpha$  on OR for PCOS detected in subgroup analyses, these results suggest the following gene-gene interactions: *PPAR* $\gamma$ 2 with *APM1*, *PGC-1* $\alpha$  with *PPAR* $\gamma$ 2, *PGC-1* $\alpha$  with *APM1*, *PGC-1* $\alpha$  with *HNF-4* $\alpha$ , and *PC-1* with *HNF-4* $\alpha$ .

## Discussion

Current evidence indicates that PCOS is a genetically complex disorder, with combined effects from multiple interacting genes (7). Therefore, we examined possible associations of single nucleotide polymorphisms and their combinations in 17 genes, reported to affect insulin sensitivity, obesity, type 2 diabetes mellitus, inflammation and/or dyslipidemia, with PCOS.

We showed that the 1059 G/C polymorphism of *CRP* increased OR for PCOS, in addition to a previously reported association of the Pro12Ala polymorphism of *PPAR* $\gamma$ 2 (18) and the Lys121Gln polymorphism of *PC-1* (19) with PCOS. Our sample size did not allow the detection of very small differences in the distribution of genotypes between PCOS and control women. Therefore, we can not reject the possibility that minor differences in the distribution of *APM1* genotypes could be detected in a larger PCOS population. Moreover, we found some previously unreported in any population gene–gene interactions between *PGC-1 $\alpha$* , *PPAR* $\gamma$ 2, *APM1*, *PC-1* and *HNF-4 $\alpha$*  and showed for the first time in PCOS subjects an interaction between *PPAR* $\gamma$ 2 and *AMP1*, detected recently in members of hypertensive families (33) and in type 2 diabetic women (34).

C-reactive protein (CRP) is a strong predictor of type 2 diabetes mellitus, cardiovascular disease and/or stroke. Women with PCOS present elevated levels of CRP (35, 36), although increased concentrations of CRP have also been related to obesity in Finnish (37), German (38) and Spanish (39) populations. There are, however, no data available to show the effect of the 1059 G/C polymorphism of *CRP* on CRP levels. Therefore, it remains to be verified whether the 1059 G/C polymorphism influences the CRP level and the risk of cardiovascular disease and type 2 diabetes among PCOS patients.

The present study indicates that multiple loci rather than a single locus analysis are required for the genetic dissection of PCOS. We detected possible interactions between variants of genes related to

adipocyte function and insulin signaling (*PGC-1 $\alpha$* , *PPAR $\gamma$ 2*, *APM1*, *PC-1* and *HNF-4 $\alpha$* ). Although the mechanism(s) of gene-gene interactions detected in this study is yet unknown, they are physiologically conceivable. PGC-1 $\alpha$  is a transcriptional factor involved in the regulation of adaptive thermogenesis through the association with PPAR $\gamma$ . The Gly487Ser polymorphism of *PGC-1 $\alpha$*  has been found to influence energy expenditure (40) and lipid metabolism (41). Moreover, the *PGC-1 $\alpha$*  gene locus is associated with altered glucose metabolism (42) and type 2 diabetes (43, 44). PPAR $\gamma$ 2 is expressed in white and brown adipose tissue and has a key role in the regulation of adipogenesis and lipid and glucose metabolism. The Pro12Ala polymorphism of *PPAR $\gamma$ 2* has been shown to be associated with insulin resistance and adiponectin levels in Japanese (45), but not in European (46) subjects. Additionally, the 161 C/T polymorphism in exon 6 of *PPAR $\gamma$ 2* has been linked to obesity in PCOS patients (17). In concordance with previous results in type 2 diabetic patients (44), we did not observe an interaction between the Gly487Ser polymorphism of *PGC-1 $\alpha$*  and the Pro12Ala polymorphism of *PPAR $\gamma$ 2*, but we detected a possible interaction between two other variants, the 3'UTR G/A of *PGC-1 $\alpha$*  and the 161 C/T of *PPAR $\gamma$ 2*. In agreement with previously reported genetic epistasis of *APM1* and *PPAR $\gamma$ 2* (33, 34), our data suggest an interaction between the Pro12Ala polymorphism of *PPAR $\gamma$ 2* and the 276 G/A polymorphism of *APM1*. Moreover, we found for the first time an interaction between the 3'UTR G/A polymorphism of *PGC-1 $\alpha$*  and the 276 G/A polymorphism of *APM1*. Therefore, we suggest that combinations of genomic variants in genes that regulate adipocyte differentiation and adipose-derived hormone levels may substantially contribute to the pathogenesis of PCOS.

To our knowledge this is the first report of an interaction between the Gly487Ser polymorphism of *PGC-1 $\alpha$*  and the IVS3 G/A polymorphism of *HNF-4 $\alpha$* . Arslanin *et al.* (47) observed increased hepatic glucose production in obese adolescents with PCOS. In addition, PGC-1 $\alpha$  interacts with HNF-4 $\alpha$  to control insulin-regulated gluconeogenesis (48). Therefore, we postulate that combinations of

genomic variants of *PGC-1 $\alpha$*  and *HNF-4 $\alpha$*  may influence hepatic insulin resistance in PCOS patients. Involvement of genomic variants in other genes related to the regulation of gluconeogenesis can not be excluded and should be further investigated.

A large body of data suggests a defective autophosphorylation of insulin receptor in PCOS (49). The Gln121 allele of *PC-1*, associated with increased OR for PCOS in our study population (19), inhibits tyrosine kinase activity of insulin receptor and has been proposed to increase insulin resistance. Impaired insulin signaling in hepatocytes may directly dysregulate hepatic nuclear factors and alter HNF-regulated transcription in liver and pancreatic  $\beta$ -cells. Thus, susceptibility to the progression to type 2 diabetes might differ between individuals carrying certain genomic variants in genes encoding hepatic nuclear factors, e.g. *HNF-4 $\alpha$* . Whether *PC-1* and *HNF-4 $\alpha$*  modify the risk for the development of type 2 diabetes in PCOS subjects remains to be determined.

The current study reports for the first time the association of the 1059 G/C polymorphism in *CRP* with PCOS. To our knowledge this is also the first study providing the evidence on gene-gene interactions in PCOS. We showed the association of PCOS with different combinations of genomic variants in *PPAR $\gamma$ 2*, *PGC-1 $\alpha$* , *APM1*, *PC-1* and *HNF-4 $\alpha$* , supporting the hypothesis that genes involved in the etiology of PCOS may act epistatically. This may also explain why positive results from studies on single polymorphisms usually do not replicate across independent samples. In conclusion, we propose that single gene effects as well as gene-gene interactions may substantially contribute to the pathogenesis of PCOS. Combinations of variants in genes related to adipocyte function and insulin signaling seem to be linked to PCOS, and require further investigations in other populations.

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**Table 1.** Genotype distribution on genes of interest in control subjects and in patients with polycystic ovary syndrome (PCOS)

Gene	Polymorphism/ SNP	Genotype distribution, % *		p for genotypes (alleles)
		controls	PCOS	
<i>PPAR</i> $\gamma$ 2	Pro12Ala	66.1 / 29.6 / 4.3	77.0 / 20.9 / 2.2	0.141 (0.045)
	161 C/T	60.0 / 37.4 / 2.6	67.1 / 32.2 / 7.0	0.285 (0.191)
<i>PGC-1</i> $\alpha$	Gly482Ser	42.6 / 45.4 / 12.0	43.6 / 46.4 / 10.0	0.878 (0.652)
	3'UTR+2390G/A	81.3 / 17.9 / 0.9	86.5 / 13.5 / 0.0	0.326 (0.298)
	rs7695542 C/T	96.3 / 3.7 / 0.0	95.8 / 4.2 / 0.0	0.823 (0.825)
<i>HNF-1</i> $\alpha$	Ile27Leu	32.4 / 45.0 / 22.5	28.2 / 52.8 / 19.0	0.470 (0.933)
	Ser487Asn	44.6 / 41.1 / 14.3	45.0 / 42.9 / 12.1	0.875 (0.769)
<i>HNF-4</i> $\alpha$	-3926 C/T	58.4 / 37.2 / 4.4	65 / 29.3 / 5.7	0.402 (0.471)
	IVS3+2925 G/A	75.5 / 22.7 / 1.8	82.5 / 16.8 / 0.7	0.336 (0.143)
<i>USF1</i>	5'UTR C/T	43.5 / 43.5 / 13.0	36.4 / 49.7 / 14.0	0.503 (0.346)
<i>CAPN10</i>	UCSNP-43 G/A	72.2 / 24.3 / 3.5	66.4 / 26.6 / 7.0	0.391 (0.176)
	UCSNP-44 T/C	72.5 / 25.7 / 1.8	67.8 / 25.9 / 6.3	0.222 (0.180)
	UCSNP-63 C/T	70.0 / 28.2 / 1.8	72.0 / 24.5 / 3.5	0.608 (0.957)
<i>CRP</i>	1059 G/C	90.3 / 8.0 / 1.8	81.1 / 18.9 / 0.0	0.014 (0.123)
<i>TNF-<math>\alpha</math></i>	-308 G/A	81.7 / 18.3 / 0.0	78.3 / 18.9 / 2.8	0.190 (0.259)
<i>IL-1</i> $\beta$	-511 C/T	32.7 / 51.8 / 15.5	42.0 / 43.4 / 14.7	0.306 (0.252)
<i>IL-6</i>	174 C/G	23.5 / 51.3 / 25.2	27.3 / 51.0 / 21.7	0.704 (0.408)
<i>IL-10</i>	-1082 A/G	25.9 / 53.6 / 20.5	29.4 / 45.5 / 25.2	0.427 (0.896)
<i>PC-1</i>	Lys121Gln	81.7 / 17.4 / 0.9	62.9 / 28.0 / 2.8	0.060 (0.017)
<i>APM1</i>	45 T/G	93.9 / 5.2 / 0.9	87.4 / 11.9 / 0.7	0.173 (0.109)
	276 G/T	46.1 / 41.7 / 12.2	53.8 / 40.6 / 5.6	0.134 (0.075)
<i>GLP1R</i>	Gly168Ser	55.8 / 39.8 / 4.4	52.4 / 39.2 / 8.4	0.443 (0.354)
<i>LIPC</i>	-250 G/A	58.3 / 31.3 / 10.4	53.8 / 39.9 / 6.3	0.239 (0.972)
<i>GLUT2</i>	Thr110Ile	70.8 / 26.5 / 2.7	76.1 / 23.2 / 0.7	0.360 (0.243)
<i>APOA</i>	Ser16Trp	90.1 / 9.9 / 0.0	87.4 / 12.6 / 0.0	0.506 (0.519)

\* Percentages for the common genotype/heterozygous/rare genotype

*PPAR* $\gamma$ 2 = peroxisome proliferator receptor gamma 2; *PGC-1* $\alpha$  = peroxisome proliferators activated receptor coactivator 1; *HNF-1* $\alpha$  and *HNF-4* $\alpha$  = hepatocyte nuclear factor 1 and 4; *USF1* = upstream stimulatory factor 1; *CAPN10* = calpain 10; *CRP* = C-reactive protein; *TNF- $\alpha$*  = tumor necrosis factor- $\alpha$ ; *IL-1* $\beta$ , *IL-6* and *IL-10* = interleukin 1 $\beta$ , 6 and 10; *PC-1* = plasma cell glycoprotein 1; *APM1* = adiponectin; *GLP1R* = glucagon-like peptide 1 receptor; *LIPC* = hepatic lipase; *GLUT2* = glucose transporter 2; *APOA* = apolipoprotein A

**Table 2.** The effect of the combination of variants of *PPAR* $\gamma$ 2, *PGC-1* $\alpha$ , *APM1*, *PC-1* and *HNF-4* $\alpha$  on odds ratios [OR] for PCOS in subgroup analyses

Risk genotype	Subgroups		OR	95% CI	p
	Gene	Variant			
<i>PPAR</i> $\gamma$ 2 : 161 C/C	<i>PPAR</i> $\gamma$ 2	Pro12Pro	1.00	-	-
		Ala12 allele	1.89	0.96-3.73	0.066
			0.43	0.16-1.20	0.106
<i>APM1</i> : 276 G/G	<i>PGC-1</i> $\alpha$	3'UTR G/G	1.00	-	-
		3'UTR A allele	1.22	0.71-2.10	0.475
			4.85	1.08-21.76	0.039
<i>HNF-4</i> $\alpha$ : IVS3 G/G	<i>PGC-1</i> $\alpha$	Gly482Gly	1.00	-	-
		Ser482 allele	1.03	0.40-2.60	0.957
			2.61	1.10-6.20	0.030
	<i>PC-1</i>	Lys121Lys	1.00	-	-
		Gln121 allele	2.53	1.17-5.44	0.018
		0.56	0.16-1.99	0.371	

In each subgroup, genotypes were encoded as:

0 = the presence of the protective genotype, 1= the presence of the risk genotype

*PPAR* $\gamma$ 2 = peroxisome proliferator receptor gamma 2; *PGC-1* $\alpha$  = peroxisome proliferators activated receptor coactivator 1; *APM1* = adiponectin; *PC-1* = plasma cell glycoprotein 1; *HNF-4* $\alpha$  = hepatocyte nuclear factor 4

**Table 3.** Gene-gene interactions between the variants of *PGC-1 $\alpha$* , *PPAR $\gamma$ 2* and *APM1* and between the variants of *PC-1* and *HNF-4 $\alpha$* , and odds ratios [OR] for PCOS in subjects with at least one risk genotype or both risk genotypes

Risk genotypes	OR	95% CI	p	Interaction term
<i>PPAR<math>\gamma</math>2</i> : Pro12Pro	1.00	-	-	p= 0.049
	1.85 <sup>a</sup>	0.90-3.77	0.093	
<i>APM1</i> : 276 G/G	2.36 <sup>b</sup>	1.08-5.17	0.032	
<i>PGC-1<math>\alpha</math></i> : 3'UTR G/G	1.00	-	-	p= 0.061
	3.56 <sup>a</sup>	0.92-13.75	0.066	
<i>PPAR<math>\gamma</math>2</i> : 161 C/C	3.89 <sup>b</sup>	0.99-15.36	0.052	
<i>PGC-1<math>\alpha</math></i> : 3'UTR G/G	1.00	-	-	p= 0.023
	1.33 <sup>a</sup>	0.79-19.30	0.281	
<i>APM1</i> : 276 G/G	5,00 <sup>b</sup>	1.30-19.30	0.020	
<i>PC-1</i> : Gln121 allele	1.00	-	-	p=0.088
	2.73 <sup>a</sup>	1.28-5.78	0.009	
<i>HNF-4<math>\alpha</math></i> : IVS3+2925 G/G	3.50 <sup>b</sup>	1.40-8.72	0.007	

Genotypes were encoded as:

<sup>a</sup>: 0 =the presence of both protective genotypes, 1= the presence of at least one risk genotype

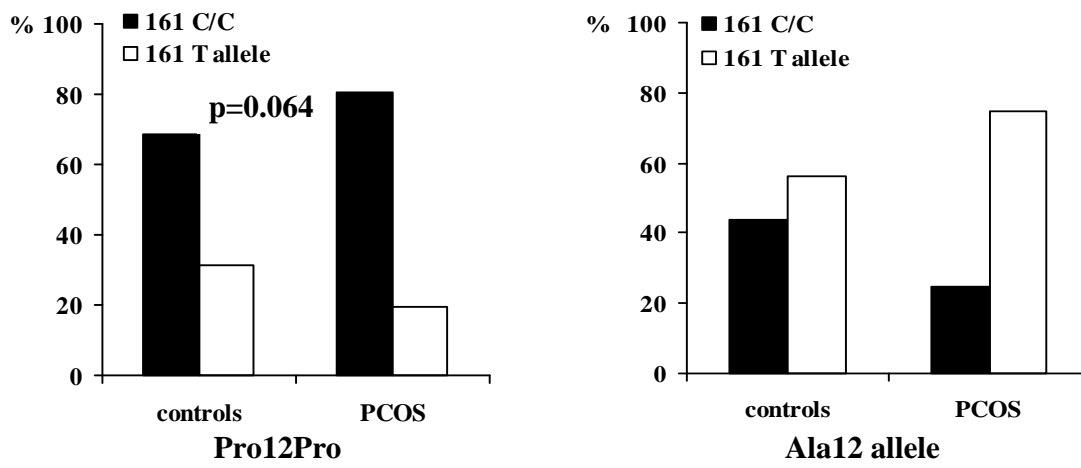
<sup>b</sup>: 0 = the presence of both protective genotypes, 1= the presence of both risk genotypes

*PGC-1 $\alpha$*  = peroxisome proliferators activated receptor coactivator 1; *APM1* = adiponectin; *PPAR $\gamma$ 2* = peroxisome proliferator receptor gamma 2; *PC-1* = plasma cell glycoprotein 1; *HNF-4 $\alpha$*  = hepatocyte nuclear factor 4

**Figure 1.** Percentage (%) of subjects with the 161 C/C genotype and the 161 T allele of *PPAR* $\gamma$ 2 in PCOS and control women according to the Pro12Ala polymorphism of *PPAR* $\gamma$ 2.

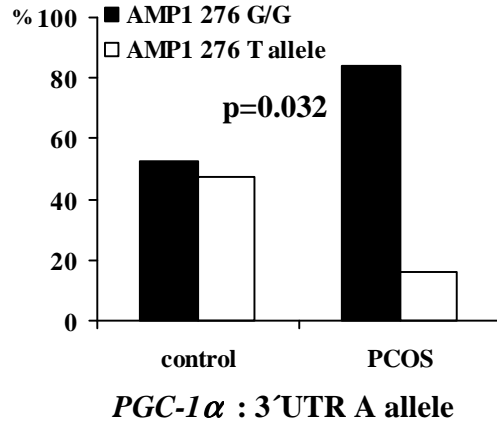
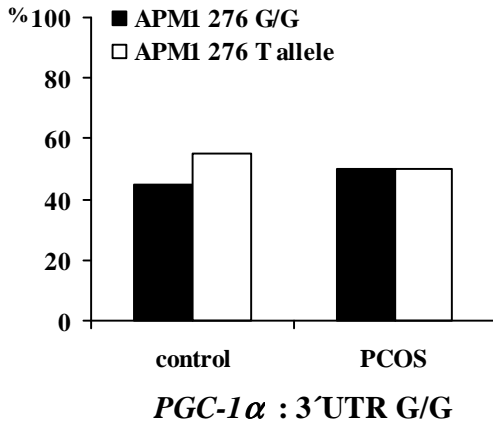
**Figure 2.** Percentage (%) of subjects with A) the 276 G/G genotype and the 276 T allele of *APM1* according to the 3'UTR G/A polymorphism of *PGC-1 $\alpha$*  B) the IVS3 G/G genotype and IVS3 A allele of *HNF-4 $\alpha$*  according to the Gly482Ser polymorphism of *PGC-1 $\alpha$* , and C) the IVS3 G/G genotype and IVS3 A allele of *HNF-4 $\alpha$*  according to the Lys121Gln polymorphism of *PC-1*, in PCOS and control women.

**Figure 1**

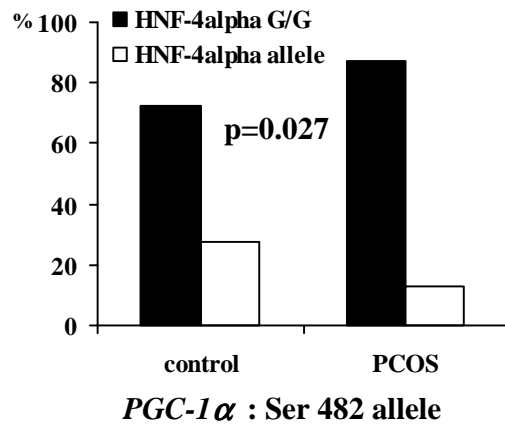
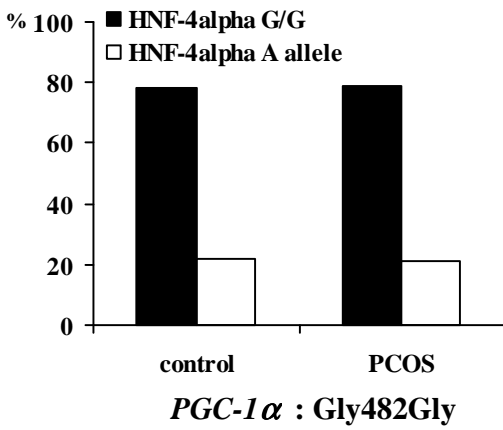


**Figure 2**

**A)**



**B)**



**C)**

